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Going forward together: cooperative invasion in melanoma

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Early-stage melanomas can be excised by surgery, but once they have begun to invade and metastasize, treatment requires additional therapeutic interventions. Thus, understanding the mechanisms that drive tumor invasion and metastasis is critical for drug development, and to prevent the spread of disease. In a recent study from the Wellbrock and Hurlstone laboratories, melanoma cells with less invasive potential are shown to become invasive with the help of other tumor cells, demonstrating that melanoma cells can work together to promote malignancy (Chapman et al., 2014). These interactions may be important drug targets.

One of the most enticing features of the zebrafish system for cancer biology is live in vivo imaging (White et al., 2013). Zebrafish embryos are optically transparent, enabling fluorescent reporter lines to reveal tissue development and cellular movements in amazing detail. Zebrafish are fertilized outside the mother, so that embryos can be imaged from the single cell stage through to adulthood. As vertebrates that share over 70% of the genome with humans, zebrafish develop cancers with many of the pathological features as humans. In addition, zebrafish are amenable to (xeno) transplantation, so that the fundamental aspects of cancer cell biology can be visualized in living animals. Their small size (10 embryos can easily fit into a well of a 24-well plate in 1 ml of solution) means that this is an ideal system to screen and test new compounds. Already, drug activity identified through zebrafish small molecule screens are in clinical

trial, including leflunomide for the treatment of melanoma (NCT01611675).

Malignant melanoma is comprised of different tumor cell subpopulations, but how these subpopulations relate to each other and contribute to cancer progression is not well understood. Multicellular imaging in cancers has revealed networks of paracrine interactions that contribute to heterogeneity and collective cell movements (Calvo and Sahai, 2011). In fact, some of the first evidence for cooperative interactions in melanoma was shown in primary melanoma explants whereby differential adhesion to matrix-enabled collective migration (Hegerfeldt et al., 2002). In a new study by Chapman et al. (2014), they take advantage of a zebrafish-melanoma xenograft model to show that heterogeneous tumor cell subpopulations can interact with each other to contribute to tumor progression via *cooperative invasion*. To examine the potential importance of melanoma heterogeneity, Chapman et al. used two melanoma cell lines, both with the common BRAF^{V600E} mutation, but with different invasive potentials (WM266-4 highly invasive; 501mel poorly invasive; both derived from human metastatic melanoma). Zebrafish embryos were injected in the pericardium with either WM266-4 cells, 501mel cells or with an equal ratio of both cell types. In all cases, the melanoma cells formed tumorlike masses. Some cells could be imaged migrating away from the pericardium tumor mass in filelike patterns. Consistent with findings in other assay systems, WM266-4 cells displayed high invasiveness, whereas 501mel cells did not invade. The surprise came in the case of heterogeneous xenografts, where the invasion potential of 501mel cells increased to levels similar to that of WM266-4 cells. This indicates that poorly invading melanoma cells can alter their behavior to actively invading when interacting with other cells in a heterogeneous environment.

While the invasion potential of 501mel cells could be stimulated by the

WM266-4 cells, closer examination revealed that most of the time WM266-4 cells were leading the files of cells. It is well known that degradation of the extracellular matrix (ECM) by matrix metalloproteases is an important feature of cancer invasion. Indeed, the authors discover that WM266-4 cells express significantly higher levels of MMPs than 501mel cells. To test the involvement of the proteolytic degradation of ECM in cooperative invasion, the team immersed the xenografted larvae in water with protease inhibitors. This had no effect on homogenous xenografts of either 501mel cells (in which there is no invasion in any case) or WM266-4 cells (these cells continue to invade). In heterogeneous xenografts, however, the inhibition caused a block of invasion in 501mel cells and a significant decrease in invasion in WM266-4 cells. Supporting these findings, they then depleted the expression of a major regulator of protease-driven invasion, MT1-MMP, in WM266-4 cells, resulting again in the suppression of cooperative invasion, but not affecting the invasion of homogenous WM266-4 cells. Thus, MMPs are critical for invasion of both cell types in the context of heterogeneous xenografts, but not in the context of homogenous WM266-4 cells alone.

How is it that... cancer cells that are usually invasive suddenly become dependent on proteases for invasion?

The authors hypothesize that 501mel cells secrete a factor that inhibits the proteolytic-independent invasiveness of WM266-4 cells. Chapman et al. use a system with WM266-4

spheroid cells embedded in collagen and cocultured with either 501mel or autologous cells in transwells, so that they are physically separated each other. They find that the exposure to the 501mel cells modulates the WM266-4 cell response to protease inhibitors: WM266-4 cells shift from a

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predominantly rounded into an elongated mode of invasion characteristic of protease-dependent migration, indicating that a diffusible factor derived from 501mels can directly act upon WM266-4 cells.

MMPs and ECM deposition can be closely coupled in cancers, and the authors show that homogenous xenografts of WM266-4, but not 501mel cells, are surrounded by ECM proteins collagen I and fibronectin. In heterogeneous xenografts, however, ECM proteins are both present and even more abundant than in WM266-4 xenografts alone. Moreover, the majority of both WM266-4 and 501mel invading cells were found along the fibronectin and collagen tracks. These ECM components appear to be in flux, and treatment with protease inhibitors leads to increasing amounts of ECM. Finally, the authors generate WM266-4 cells with low levels of fibronectin by siRNA and show that WM266-4-derived fibronectin is essential for cooperative invasion. The authors conclude that it is the WM266-4 cells that are generating the ECM and that a soluble factor secreted by the 501mel cells stimulates the production and dependence on MMP–ECM proteins for WM266-4 cell invasion.

Many questions now remain to be addressed, including how cooperative invasion compares with collective cell cancer invasion described within invading tumors (Calvo and Sahai, 2011; Friedl et al., 2012; Hegerfeldt et al., 2002). It remains to be tested if the cooperative behaviors observed here

are reflections of those observed in collective cell migrations and if this is happening within a tumor in vivo (e.g., as described in Hegerfeldt et al., 2002). Given the heterogeneity within cancers (and cancer cell lines), it may be possible to identify leaders and followers within a single cancer that demonstrate similar behaviors. While the authors diligently test another two cell lines and observe generally similar behaviors, it is not clear how broadly applicable this is to other cell lines, or even between matched cell lines derived from the same cancer at different stages of cancer progression. Are there combinations of cells that do not cooperate, and what are the consequences of heterogeneous xenografts with cells of similar phenotypes? An important step will be to determine markers of the different cell types that contribute to cooperative invasion (it does not seem to be *MITF* expression). Mechanistically, future work should address the very interesting questions of how non-invasive cells cause invasive cells to lose their invasive non-protease-dependent activity, and why fibronectin deposition impairs cooperative movement in the presence of MMP inhibition but supports invasion without MMP inhibition. Finally, with a wide range of transgenic reporter lines in zebrafish, the authors are now well poised to address the role of host-derived factors, such as fibroblasts, macrophages, and neutrophils, in cooperative invasion.

One of the most important findings is that the cooperative cross talk between cells is targetable. If indeed, this pro-

cess is important in vivo, the authors have a powerful in vivo zebrafish assay to screen for drugs and drug-leads that can target this activity, and to capture the live imaging of the cell behaviors in a whole animal context. As proof-of-principle, the authors have already shown that protease inhibitors can inhibit cooperative invasion. Given the dangerously high-invasive and migratory behaviors of melanoma, it is important to have new targetable processes for this disease.

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